RESEARCH ARTICLE

Combination of Fibrinogen and High-sensitivity C-reactive Protein Measurements is Potential in Identification of Acute Coronary Syndrome

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Abstract

BACKGROUND: Acute myocardial infarction (AMI) is one of cardiovascular diseases with high morbidity and mortality rates. Novel biomarkers that can detect accurately acute coronary syndrome (ACS) at early stage, are necessary to improve current strategies and/or to identify subjects who are at risk. Fibrinogen and high-sensitivity C-reactive protein (hs-CRP) roles in inflammation process could be potential for ACS early detection. This study was conducted to evaluate measurements of fibrinogen and hs-CRP on ACS.

METHODS: An analytic observational study with cross sectional approach was conducted on patients with Troponin I positive. After signing informed consent, anamnesis and complete blood count were conducted. Besides that, liver function, renal function, and blood glucose tests were conducted as well. Samples of selected subjects were quantified with enzyme-linked immunosorbent assay (ELISA) for Troponin I, fibrinogen and hs-CRP. Then statistical analyses were performed.

RESULTS: There were 76 subjects in each ACS and non-ACS groups. ACS group showed significant higher levels of both fibrinogen and hs-CRP compared to Non-ACS group (p=0.000). Among evaluated risk factors, diabetes mellitus (DM) (p=0.003) and hypertension (p=0.000) were significantly higher in ACS group than in non-ACS group. Among evaluated clinical factors, blood glucose (p=0.001) and age (p=0.000) were significantly higher in ACS group than in non-ACS group. Combination of fibrinogen and hs-CRP measurements showed the highest sensitivity (75.00%), specificity (80.26%), accuracy (77.63%), positive predictive value (79.19%) and negative predictive value (76.25%).

CONCLUSION: Since fibrinogen and hs-CRP were increased in ACS group and combination of fibrinogen and hs-CRP measurements showed the highest sensitivity, specificity, accuracy, positive predictive value and negative predictive value, we suggest that combination of fibrinogen and hs-CRP measurements could give added value to identify ACS.

KEYWORDS: fibrinogen, hs-CRP, biomarker, ACS, acute coronary syndrome, atherosclerosis, inflammation

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Introduction

Acute myocardial infarction (AMI) is one of cardiovascular diseases (CVDs) with high morbidity and mortality rates. American Heart Association estimated that more than 6 millions Americans have coronary heart disease (CHD) and it is estimated that 1 million have myocardial infarction attack every year. CHD is also the leading cause of death (20%) in United States of America. Indonesian Domestic

Health Survey / *Survei Kesehatan Rumah Tangga* (SKRT) performed in 1992, showed that people over 40 years old have CVD-caused mortality at the first rank (16%). Similar survey held in Java and Bali in 1995 pointed that CVD-caused mortality was at the first rank as well, and its percentage (25%) was higher compared to the data from SKRT in 1992. Among CVD, CHD had the highest morbidity rate (30-36.1%).(1)

High AMI morbidity and mortality rates suggested the necessity of an earlier diagnosis for immediate treatment and death prevention. Acute coronary syndrome (ACS) is a heart emergency situation of which clinical manifestation is chest discomfort or any other symptoms due to myocardial infarction. ACS consists of unstable angina pectoris. AMI is divided into ST segment elevation myocardial infarction (STEMI) and non STEMI (NSTEMI).(2,3)

To date, ACS diagnosis was determined by cardiac enzyme Troponin I or T measurements that could be detected started from 4 to 8 hours after the onset of chest pain. Troponin reached its peak level at 12-36 hours after the onset, then kept increasing for 7-9 days. False positive was commonly found in patients with chronic kidney disease (CKD) and those who were undergoing dialysis. Another biomarker for ACS is creatine kinase muscle brain that could be detected 4-6 hours after AMI onset and reaches its peak level within 18-24 hours then back to its normal value in 2-3 days. This biomarker is not quite specific for cardiac muscle and false positive was sometimes found in patients with renal failure.(4,5)

Fibrinogen and C-reactive protein (CRP) are biomarkers that act as acute phase proteins. Fibrinogen plays role in coagulation process, blood viscosity, and platelet aggregation, while high sensitivity CRP (hs-CRP) works in inflammation process. Many studies have reported fibrinogen and hs-CRP used as an early stage detecting biomarker and prognosis decision for CHD.(6,7) If these two biomarkers were detected earlier in ACS patients, the proper treatment can be immediately given, and more extensive myocardial muscle damage can be prevented. (8,9) Currently, in Indonesian hospitals, particularly in Dr. Saiful Anwar Hospital in Malang, the fibrinogen and hs-CRP measurements have not yet been considered as a routine procedure for ACS patients. Enzyme evaluation that is usually performed tends to reflect that there has been cardiac muscle damages.(2,10,11) Meanwhile, the role of inflammatory marker in ACS diagnosis has been reported.(12-14) Fibrinogen and hs-CRP roles in inflammation process indicate that fibrinogen and hs-CRP can be more potential biomarkers for ACS early detection, compared to other current biomarkers. This study evaluated measurements of fibrinogen and hs-CRP on ACS.(6,15,16)

Methods

Study Design and Subjects Recruitment

This study was an analytic observational with cross sectional approach to evaluate the diagnostic value of fibrinogen and hs-CRP in establishing ACS diagnosis among patients hospitalized in Dr. Saiful Anwar Hospital with Troponin I positive. Subjects were men and women aged 35 to 80 years old with ACS as the case group and without ACS as the control group. Subjects with severe infection, liver function disorder, renal function disorder, and malignancy were eliminated from this study. This research was carried out on patients from emergency department and cardiovascular intensive care unit of Dr. Saiful Anwar Hospital.

Data Collection, Sample Preparation and Enzyme-linked Immunosorbent Assay (ELISA)

After signing informed consent, proper anamnesis was conducted. Then blood sample was collected for complete blood count. Besides that, liver function, renal function, and blood glucose tests were all conducted. Samples of selected subjects were quantified with enzyme-linked immunosorbent assay (ELISA) for Troponin I (Troponin I ELISA Kit, Sigma Aldrich, St. Louis, MO, USA), fibrinogen (Fibrinogen Human ELISA Kit, Abcam, Cambridge, UK) and hs-CRP (Quantikine® ELISA Human C-Reactive Protein/CRP Immunoassay, Minneapolis, MN, USA).

Statistical Analysis

Data was descriptively analyzed to portray the characteristics of case and control groups, as well as the information of classic risk factors and clinical characteristics. Differences of fibrinogen and hs-CRP in both groups were statistically examined with independent t-test. Correlation test was performed as well. To find out which biomarker level has the highest accuracy to ACS, cut-off point for each level and logistic regression were carried out to find the predicted probability value for fibrinogen combination data and hs-CRP as the basis of cut-off value determination through receiver operating curve (ROC). The sensitivity, specificity, likelihood ratio and predictive value were further counted by using fibrinogen and hs-CRP, either separated or combination of both.

Results

There were 76 subjects in each group, hence there were totally 152 subjects of men and women. The mean of age was 57.5 years old. Comparison of fibrinogen and hs-CRP levels between ACS and non-ACS groups is shown in Table 1. ACS group had higher levels of both fibrinogen and hs-CRP compared to Non-ACS group. Independent t-tests for fibrinogen and hs-CRP levels between ACS and non-ACS group were significant (p=0.000, p<0.05).

Meanwhile, systolic and diastolic BP did not have significant differences between ACS and Non-ACS groups (p>0.05).

Cut-off for hs-CRP was set at 3.05 mg/L, while cut-off for fibrinogen was 4.25 g/L. More subjects with higher hs-CRP level (hs-CRP≥3.05 mg/L) were found in ACS group (69.7%) (Table 4). More subjects with higher fibrinogen level (Fibrinogen ≥ 4.25g/L) were found in ACS group as well (72.4%). When hs-CRP cut-off was combined with fibrinogen cut-off, more subjects with higher hs-CRP and fibrinogen levels were found in ACS group (80.3%).

Table 1. The Comparison of Fibrinogen and hs-CRP Levels between ACS and Non-ACS Groups.

Biomarker	Group		*p* value*
	Non ACS (Mean ± SD)	ACS (Mean ±SD)	
Fibrinogen (g/L)	3.78 ± 1.38	5.08 ± 1.56	0.000
hs-CRP (mg/L)	1.95 ± 1.25	4.04 ± 1.94	0.000

*: Independent t-test

Characteristics of classic risk factor in ACS and non-ACS groups are shown in Table 2. The evaluated risk factors were cigarette smoking, diabetes mellitus (DM), history of hypertension and ACS family history. DM was significantly higher in the ACS group than in non-ACS group. Hypertension was also found significantly higher in ACS group. Meanwhile, cigarette smoking behavior and ACS family history were also higher in ACS group, however the values were not significant (p>0.05).

Clinical characteristics in ACS and Non-ACS groups are shown in Table 3. The evaluated clinical factors were systolic blood pressure (BP), diastolic BP, blood glucose and age. Not all clinical characteristics had significant differences between those two groups. The significant differences were found in blood glucose and age (p<0.05).

In combination, fibrinogen and hs-CRP showed the highest sensitivity (75.00%) (Figure 5). The same results were also found in specificity (80.26%), accuracy (77.63%), positive predictive value (PPV) (79.17%), and negative predictive value (NPV) (76.25%).

Discussion

Our current results shows that detection of both fibrinogen and hs-CRP could provide high sensitivity, specificity, accuracy, PPV and NPV results for ACS detection. Since fibrinogen and hs-CRP are related with ACS pathophysiology in early stage, these biomarkers could be useful for early detection of ACS.(17-19) Troponin I detection has higher sensitivity

Table 2. Characteristics of Classic Risk Factor in ACS and Non-ACS Groups.

Risk Factor	Group				*p* value
	Non ACS (n=76)		ACS (n=76)		
	n	%	n	%	
Cigarette Smoking	27	35.5	35	46.1	0.248
DM	11	14.5	28	36.8	0.003*
Hypertension	11	14.5	37	48.7	0.000*
ACS Family History	0	0	4	5.3	0.120

*: Pearson Correlation Test, significant (p<0.05)

and specificity than fibrinogen and hs-CRP. However, when Troponin I is detected, it will be ACS late stage, the cardiac muscle has undergone necrosis or irreversible death. Meanwhile fibrinogen and hs-CRP could be detected earlier, therefore, it could support early ACS treatment.(9,17)

Elevated inflammatory markers reflect acute phase response. An epidemiologic study has shown that levels of several inflammatory markers elevated few years prior to ACS manifestation.(9,17) Increased levels of fibrinogen and hs-CRP in ACS without myocardial infarction correlated to the extent and severity of atherosclerosis, while CRP level in AMI correlated to the extent of tissue damage. Various researches stated that inflammation is an integral part of ACS. CRP is detected in damaged blood vessel or myocardium, and involves in inflammation process and thrombosis through complement activity. CRP is also directly related to tissue factor expression over monocyte surface.(3,20)

Fibrinogen and CRP levels are correlated to several classic risk factors like obesity, cigarette smoking, blood pressure and blood glucose. Healthy people, whose parents have AMI or ACS, can have higher CRP level compared to those without AMI family history. Higher fibrinogen and hs-CRP levels are reported more in subjects with multiple classic risk factors than in those with single classic risk factor.(20,21) Among patients with stable and unstable

Table 3. Clinical Characteristics in ACS and Non-ACS Groups.

Clinical Factor	Group		*p* value*
	Non ACS (Mean ± SD)	ACS (Mean ±SD)	
Systolic BP	126.88±111.30	132.33±29.25	0.065
Diastolic BP	81.30±8.32	78.19±16.84	0.332
Blood Glucose	149.28±90.5	189.83±120.13	0.001*
Age	50.04±8.77	59.46±11.74	0.000*

*: Pearson Correlation Test, significant (p<0.05)

Table 4. Distribution of hs-CRP and Fibrinogen Level, Based on Cut-off Point, in ACS and Non-ACS Groups.

Biomarker & Cut-Off	Group			
	Non ACS (n=76)		ACS (n=76)	
	n	%	n	%
hs-CRP<3.05 mg/L	54	71.1	23	30.3
hs-CRP≥3.05 mg/L	22	29	53	69.7
Fibrinogen< 4.25g/L	55	72.4	21	27.6
Fibrinogen ≥ 4.25g/L	21	27.6	55	72.4
hs-CRP<3.05 mg/L and Fibrinogen <0.480	57	75	15	19.7
hs-CRP≥3.05 mg/L and Fibrinogen ≥0.480	19	25	61	80.3

Table 5. Predicted Probability of Test Result.

Biomarker	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)
hs-CRP	71.05	69.74	70.39	70.13	70.67
Fibrinogen	72.37	72.37	72.37	72.37	72.37
Fibrinogen & hs-CRP	75.00	80.26	77.63	79.17	76.25

angina pectoris, it has been proven that fibrinogen and CRP can be the predictor of future AMI incidence and heart disease-caused death.(9)

Response-to-injury hypothesis states that atherosclerosis is an inflammation disease. It is marked by the presence of inflammation process, endothelial cells and smooth muscle cells, release of cytokine and growth factor, complement activation and deposition, as well as elevated plasma protein level which is called acute phase reactant. (4,5) Inflammation process that involves macrophage, lymphocyte, proteinase, and cytokine activations, promotes the thrombosis plaque rupture.(6,9) The inflammatory cells are responsible for plaque destabilization by changing anti-adhesive and anti-coagulant into pro-coagulant resulting tissue factor in monocyte to cause plaque rupture. Therefore, evident of leukocytosis and elevated CRP level indicate AMI. In 15% of AMI patients, CRP elevation is reported although Troponin T remains negative. Haidari, *et al*. investigated the correlation between serum CRP and CHD with angiography in 450 individuals. The result shows that serum CRP was higher in individual with CHD than in control group (2.14 mg/L *vs*. 1.45 mg/L) and the correlation indicated that there was inflammatory process in CHD.(9)

Previous studies demonstrate that fibrinogen could be detected in the areas of connective tissue, thrombus and cholesterol crystal loss, hence, elevated fibrinogen correlates to the degree of atherosclerosis. Fibrinogen level elevates significantly in most ACS patients, observed among 49 patients with stable angina and positive Troponin I level *vs*. stable angina with negative Troponin I level (3.87±1.2 *vs*. 3.26±0.65, p=0.02). Significant correlation between fibrinogen and Troponin I among unstable angina patients, was discovered.(12) Ben Khalfallah, *et al.*, reported that higher fibrinogen level was found in ACS patients compared to healthy individuals (4.7±1.81 g/L *vs*. 3.93±1.69 g/L, p=0.02).(6)

Our results show that combination of both markers, hs-CRP and fibrinogen provided higher specificity and sensitivity. The fibrinogen and hs-CRP are factors those synergistically correlated to ACS pathophysiology. The hs-CRP influences through inflammation phase, causing endothelial dysfunction and unstable plaques.(21) In sequence, fibrinogen takes part to the pathophysiology of ACS after plaque ruptured from coronary blood vessel by inducing coagulation cascade activation with the formation of fibrin fibers as the final process. In which if the fibers bond to platelet, thrombus would eventually formed, thus promoting ACS.(5,9,17)

Since fibrinogen and hs-CRP measurements could provide high sensitivity, specificity, accuracy, PPV and NPV results for ACS detection, these measurements could give added value to identify ACS for patients with risk factor, especially DM and hypertension. Although there are non-technical problems related to cost and equipment availability, we suggest that these measurements should be applied as routine biomarkers to enrich current diagnostic tool.

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